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Testing for the myth of cognitive reserve: Are the static and dynamic cognitive reserve indexes a representation of different reserve warehouses?

Laura Serra[a], Laura Petrosini[b], Andrea Salaris[a] Lorenzo Pica[a], Michela Bruschini[a], Carlotta Di Domenico[a], Carlo Caltagirone[c], Camillo Marra[d], Marco Bozzali[a,e]

[a]Neuroimaging Laboratory, Santa Lucia Foundation, IRCCS, Via Ardeatina, 306, 00179, Rome, Italy.

[b]Laboratory of Experimental and Behavioural Neurophysiology, IRCCS Fondazione Santa Lucia, Rome, Italy.

[c]Department of Clinical and Behavioural Neurology, Santa Lucia Foundation, IRCCS, Via Ardeatina, 306, 00179, Rome, Italy.

[d]Institute of Neurology, Catholic University, Largo Agostino Gemelli, 8, 00168, Rome, Italy.

[e]Brighton & Sussex Medical School, CISC, University of Sussex, Brighton, Falmer

East Sussex BN1 9RR, UK.

Running title: Static and Dynamic cognitive reserve in a-MCI and AD

***Correspondence to:**

Dr. Laura Serra, Via Ardeatina 306, 00179 Rome, Italy. Telephone number: #39-06-5150 1547;

Fax number: #39-06-5150 1213; Email address: l.serra@hsantalucia.it

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Abstract

Background: Cognitive reserve (CR) explains the individual resilience to neurodegeneration. Years of formal education express the static measure of reserve (sCR). A dynamic aspect of CR (dCR), has been recently proposed. Aim of the study was to compare sCR and dCR indexes respectively, to detect brain abnormalities in AD patients.

Methods: 117 individuals (39 AD, 40 a-MCI, 38 HS) underwent neuropsychological evaluation and a 3T-MRI. T1-weighted volumes were used for manual segmentation of the hippocampus and of the parahippocampal cortices. Years of formal education were used as an index of sCR. Partial Least Square analysis was used to decompose the variance of individual MMSE scores, considered as a dCR index. In a-MCI and AD patients the brain abnormalities have been assessed comparing individuals with high and low levels of sCR and dCR in turn. Moreover, we investigated the effect of the different CR indexes in mediating the relationship between changes in brain volumes and memory performances. **Results**: sCR and dCR indexes classified differently individuals having high or low levels of CR. Smaller hippocampal and parahippocampal volumes in high dCR patients were found. The sCR and dCR indexes mediated significantly the relationship between brain abnormalities and memory in patients. **Conclusions**: CR mediated the relationship between brain and memory dysfunctions. We hypothesised that sCR and dCR indexes are a representation of different warehouses of reserve not operating in parallel but forming a complex system, in which crystallised cognitive abilities and actual cognitive efficiency interact with brain atrophy impacting on memory.

Keywords:

Dynamic and static cognitive reserve; Mild Cognitive Impairment; Alzheimer's Disease, hippocampus, parahippocampal gyrus

Introduction

In the last thirty years the scientific literature has been increasingly interested in the mechanisms underlying brain and cognitive reserve [1-2]. In fact, several animal and human studies showed the beneficial effects of stimulant life experiences on the structure and functionality of the brain [2-4]. In accordance with these observations, the idea has been developed that an enriched brain, that is a brain that modified its neuronal structure as a consequence of complex environmental stimulations, better tolerates the neuronal damage [1-2]. This view assumes the development of cerebral reserves (the brain reserve-BR, the cognitive reserve-CR and finally, the neural reserve-NR) allowing a higher efficiency of the brain networks as well as a more tuned engagement of different neural pathways despite the cerebral damage [5].

Briefly, the BR refers to the brain structure (the quantity of neurons, synapses, and dendrites) supposing that subjects with larger brain cope better with the neurological damage than those with smaller brains [1-2]. The CR refers to the efficiency of cognitive functions assuming that individuals with higher level of CR are able both to use more efficiently the pre-existent cognitive processes and they are, also, able enlisting the alternative cognitive functions to withstand brain damages [1-2]. Finally, the NR refers to the efficiency of brain networks, hypothesizing that subjects with higher NR engage different brain pathways increasing the efficiency of the cognitive functions to cope the cerebral damage [1-2].

However, the identification of the best proxies' measures to assess the development of the reserves (BR, CR and NR) needs to be clarified. Currently, two kinds of measures are typically used in the studies on the CR. The static indexes, such as education years or occupational attainment, are invariant, stable along life-span [2]. Despite they are not directly related to cognitive functioning, the static indexes are the indexes most frequently used, being simple to manage in the research setting. The static

indexes reflect crystallised cognitive ability that is intellectual ability learned or achieved over time increasing the ability to gain knowledge and experience. This is something that the subject is not born with, but rather is an ability learned throughout life experiences.

Several studies reported that subjects with memory dysfunctions and higher level of CR, as measured by education years, developed the clinical symptoms of dementia (typically the Alzheimer's disease, AD) later in time than subjects with lower CR level [6-9]. Moreover, neuroimaging studies reported different structural and functional modifications of brain structures in patients with different levels of CR [5,7-8,10]. In particular, structural studies reported that patients with higher level of CR needed to accumulate more atrophy in the brain regions critical to develop AD before the symptoms of disease appeared. Typically, these regions include the hippocampus and parahippocampus [7, 11-13]. Even neuroimaging studies revealed functional connectivity changes in patients in the AD continuum with different CR levels. In particular, our recent network-based study showed both impaired and increased functional connectivity in different brain networks of amnestic Mild Cognitive Impairment (a-MCI) patients with high CR compared to a-MCI patients with low CR, while no evidence of CR effect on brain functional connectivity in AD patients and healthy elderly was evidenced [5]. More recently, dynamic CR (dCR) indexes have been introduced [10, 14-16]. These measures are sensitive to the cognitive changes due to aging and typically they are conceptualized as the residual cognitive abilities (i.e. memory, general cognitive efficiency, executive functions, etc.), after the confounding factors (such as demographic and brain variables such as cerebral atrophy or vascular lesions) have been removed [10, 14-16]. In a recent study we showed the ability of dCR indexes to single out patients with AD from patients with a-MCI [5]. More specifically, the study compared two different dCR indexes, one including the residual variance due to memory function only, and the other one including the residual variance due to both memory and general cognitive efficiency. The latter showed higher sensitivity, sensibility and accuracy to correctly classify patients of different groups [5]. The general

cognitive efficiency, estimated in terms of premorbid intelligent quotient and literacy have been previously considered as measure of CR [2]. Actually, they should be considered as static measures because they are related to cognitive ability acquired before the onset of the neurodegenerative disease. However, since we explored the cognitive efficiency measured during the course of the AD, considering it a residual measure after the effects of brain changes have been removed, even the current cognitive efficiency can be considered as a dynamic index of CR. A recent post-mortem study [17] showed that the dynamic index was a better measure of CR than the static index and that the relationship between CR and cognitive efficiency was strictly related to the presence of amyloid-plaques and neurofibrillary tangles. For the best of our knowledge, no study directly compared the ability of different kinds of CR indexes to detect brain differences in patients with AD at different disease stages *in-vivo*. In particular, we were interested to verify whether sCR respect to dCR indexes were more able to capture volumetric changes in the medio-temporal lobes (MTL), a structure considered critical for AD pathophysiology. Specifically, the hippocampus and the cortices of the parahippocampal gyrus (the perirhinal, entorhinal, and parahippocampal cortex) are early damaged in AD. Indeed, the trans-entorhinal/entorhinal cortices (Braak & Braak stage I-II) are precociously affected by the neurodegenerative processes (atrophic changes) of AD, followed by the perirhinal and parahippocampal cortices (Braak & Braak stage I-II), and then by the hippocampus (Braak & Braak stage III-IV) [18].

Automated or manual segmentation methods have been proposed to assess volumetric brain changes [19-24]. In the literature high reliability between automated and manual segmentation of the hippocampus has been described [25], while less agreement has been found for the automated and manual segmentation of the parahippocampal cortices [25]. In fact, the parahippocampal cortices show a high individual variability, and therefore automated methods are not able to completely capture this heterogeneity. In contrast, the application of the manual segmentation protocols may be more useful to

assess individual differences [25].

In the literature are present several indexes to assess the sCR, such as those derived by the CRI-q [26] or by the leisure activities questionnaire previously used by Serra and co-workers [9]. However, in these kinds of instruments the CR is a composite measure derived by several factors, such as years of formal education, occupational attainment and leisure activities. We showed [9] the ability of a composite measure of CR to detect the risk to develop AD in patients with a-MCI in association with brain abnormalities. Conversely, in the present paper we are interested to assess specifically the effect of years of formal education because it is the most frequently used measure of sCR.

In particular, the present study was aimed at investigating the ability of sCR and dCR indexes to detect volumetric changes in the MTL structures in patients with AD and a-MCI. Moreover, we assessed whether sCR and dCR indexes showed a different effect in mediating the relationship between MTL volumetric changes and memory performances.

Material and Methods

Subjects

A cohort of 117 participants, 39 with a diagnosis of probable AD, 40 with a diagnosis of amnestic MCI (a-MCI), and 38 healthy elderly subjects (HS), was enrolled. The diagnosis of probable AD was made according to the clinical criteria of the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) [27]. The patients had to respond to the diagnostic criteria for major cognitive disorder [28]. The diagnosis of a-MCI was performed according to current criteria [29] and the patients could be affected in either single (n=25) or multiple (n=15) domains. Patients with a-MCI had not to respond to the diagnostic criteria for major cognitive disorder [27], showing a CDR [30] score not exceeding 0.5. To be included in the study, healthy elderly subjects (HS) had no evidence of cognitive

impairment (see below the Neuropsychological assessment section).

As detailed below, MTL atrophy was assessed in all subjects to confirm that a-MCI and AD patients, in turn, had an intermediate likelihood of underlying AD neuropathology according with current criteria [27,29]. Healthy elderly subjects showing the presence of significant MTL atrophy were excluded. All recruited subjects with a Hachinski score [31] higher than 4 were excluded. Major systemic, psychiatric and other neurological illnesses were also carefully investigated and excluded in all participants. Finally, subjects had to be right-handed, as assessed by the Edinburgh Handedness Inventory [32] to reduce the variability due to the different hemispheric dominance that affects the organization of cognitive functions.

The principal demographic and clinical characteristics of all participants are summarized in Table 1, panel A.

The study was approved by the Ethical Committee of Santa Lucia Foundation and written informed consent was obtained from all participants before study initiation. All procedures performed in this study were in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Neuropsychological assessment

All participants underwent an extensive neuropsychological battery including the following tests :Verbal episodic long-term memory: 15-Word List (Immediate and 15-min Delayed recall) [33]; Short Story test (Immediate and 20-min Delayed recall) [34]; Visuo-spatial episodic long-term memory: Complex Rey's Figure (Immediate and 20-min Delayed recall) [34]; Short-term memory: Digit span and the Corsi Block Tapping task forward and backward [35]; Executive functions: Phonological Word Fluency [33] and Modified Card Sorting Test [36] ; Language: Naming objects

subtest of the BADA ("Batteria per l'Analisi dei Deficit Afasici", Italian for "Battery for the analysis of aphasic deficits") [37]; Reasoning: Raven's Coloured Progressive Matrices [33]; Constructional praxis: Copy of simple drawings [33] and Copy of drawings with landmarks [33]; Copy of Complex Rey's Figure [34].

For the specific purpose of the present study neuropsychological tests were not adjusted for age, gender and education, but all these demographic variables were used as covariates of no interest in the analyses.

Performances at neuropsychological tests were assessed by using seventeen ANCOVAs (with age, gender and education years as covariates of no interest). In particular, for each neuropsychological test, we compared across groups patients with AD *vs.* patients with a-MCI *vs.* HS, then we compared subjects with high or low CR indexes (static and dynamic, separately) within diagnostic groups. To avoid the type-I error Bonferroni's correction was applied (p value threshold $\alpha = 0.05/17 = 0.003$).

CR indexes computation

Static CR index

As shown in Figure 1 panel A, to compute the static CR index (sCR) in each participant we used the years of formal education. As previously reported [5,7], we divided participants on the basis of their level of formal education. Within each group, the years of formal education were transformed in z scores. Mean (μ) and standard deviation (σ) of years of formal education was first estimated in each sample. Then, for each subject, a z score representative of the individual level of formal education was calculated as follows:

$$z = (x - \mu) / \sigma$$

where x is the raw score (years of formal education) to be standardized.

Individuals reporting a z score ≤ 0 were considered having low static cognitive reserve (L_sCR).

Conversely, individuals with a z score > 0 were considered having high static cognitive reserve (H_sCR). Table 1 panel B summarizes the principal characteristics of all subjects divided according their sCR level.

Dynamic CR index

To obtain the dynamic CR index (dCR) we applied a modified version of the statistical procedures illustrated in Serra et al., 2017 [10]. In particular, as shown in Figure 1 panel B, in each participant we used the raw score of the Mini Mental State Examination (MMSE) [38] as measure of general cognitive efficiency. Assuming that demographic and brain features are independent variables (X) that may affect the MMSE score (dependent variable Y) (Figure 1, panel B^1), we first assessed the potential correlations between all considered variables by using linear correlation analyses (Figure, 1 panel B^2), then we used the Partial Least Square (PLS) analysis to estimate the covariance between MMSE score (Y) and the independent variables (Xs) that might explain part of the MMSE score variance. For a detailed description of PLS see Serra and co-workers [10]. Briefly, PLS is a statistical method used when many manifest and collinear factors can be hypothesized but only few underlying (named latent factors) account for most of the variation in the response. PLS extracts these latent orthogonal factors (that are part of the variance of the X). In the present case demographic variables (age, gender and years of formal education) and the hippocampal atrophy, as measured by the Medial Temporal lobe Atrophy (MTA) scale [39] entered in the PLS analysis as independent variables. Consequently, variance in the MMSE score (dependent variable) was decomposed into orthogonal latent factors. The minimum number of latent factors (named latent scores, LTs) explaining the maximum covariance of MMSE score was retain for further analyses. Moreover, the Variable Importance in the Projection index (VIP index) was used to assess the contribution of each considered variable in the composition of MMSE score variance into the latent scores. Then, the variables showing the highest VIP (VIP$\geq$1) were

regressed from the latent scores using the linear regression model (Figure 1, panel B^4). The standardised residual value of variance in MMSE score, remaining after accounting for all nuisance variables, was considered as an index of dynamic CR (dCR) (Figure 1, panel B^5). Moreover, to verify the independence of dCR index as new measure of reserve linear correlation analyses (Figure 1, panel B^6) were performed between dCR index and the demographical and brain variables (Figure 1, panel B^7). As for the static index subjects reporting a z score ≤ 0 were considered having low dynamic cognitive reserve (L_dCR). Conversely, individuals with a z score > 0 were considered having high dynamic cognitive reserve (H_dCR) (Figure 1, panel B^8). Table 1 panel C summarizes the principal characteristics of all subjects divided according their dCR level.

Insert Figure 1 around here

Finally, correlations between sCR and dCR indexes were calculated by using Pearson's coefficient in each group separately.

Statistical analyses were carried out in SPSS 21 (SPSS Inc., Chicago, Illinois).

MRI acquisition

All participants underwent an MRI examination at 3T (Magnetom Allegra, Siemens, Erlangen, Germany), including the following acquisitions: 1) dual-echo turbo spin echo [TSE] (TR=6190 ms, TE=12/109 ms); 2) fast-fluid attenuated inversion recovery (FLAIR) (TR=8170 ms, TE=96 ms, TI=2100 ms); 3) 3D-Modified Driven Equilibrium Fourier Transform (MDEFT) scan (TR=1338 ms, TE=2.4 ms, Matrix=256x224, n. slices=176, thickness=1 mm). According to the inclusion criteria, TSE and FLAIR scans were reviewed to exclude the presence of remarkable macroscopic brain abnormalities, as previously described [40].

Medial temporal lobe atrophy

The Medial Temporal lobe Atrophy scale (MTA) [39] was employed on MDEFT images to assess the severity of atrophy in each subject. This scale provides a rating score from 0 to 4, with scores $\geq$ 1.5 [39] indicating significant atrophy. For each subject we averaged the scores obtained in the right and left hemispheres to obtain a single measure of medial temporal lobe atrophy. One-way ANOVA was employed to control for between- (AD *vs.* a-MCI *vs.* HS) and within-group differences (L_{sCR} *vs.* H_{sCR}; L_{dCR} *vs.* H_{dCR}, respectively).

Volumetric assessment of the medial temporal lobe structures

None of the MDEFT volumes from all subjects was affected by macroscopic artefacts, as assessed by visual examination. In order to measure the volumes of the hippocampi and perirhinal, entorhinal, and parahippocampal cortices in the parahippocampal gyrus, on each MDEFT image we applied the manual segmentation protocols according to Pruessner's and Insausti's guidelines [41-43]. Firstly, each MDEFT image was warped to the T1-weighted MNI atlas (available in FSL), using the FMRIB's Nonlinear Image Registration Tool (FNIRT) (/fsl.fmrib.ox.ac.uk/fsl/fslwiki/FNIRT/). The ROIs for the bilateral hippocampus and bilateral cortices into the parahippocampal gyrus (perirhinal, entorhinal and parahippocampal cortices) were mapped using the interactive program MANGO (http://ric.uthscsa.edu/mango/).

In order to adjust for the effect of brain atrophy, in each subject we normalised the volumes of the hippocampus and the cortices of the parahippocampal gyrus for the global grey matter volume and for the length of the collateral sulcus, respectively. MDEFT volumes were pre-processed using the VBM protocol implemented in SPM8 (http://www.fil.ion.ucl.ac.uk/spm/), which consists of an iterative combination of segmentations and normalizations to produce a GM probability map [44-45] in standard space (Montreal Neurological Institute, or MNI coordinates) for every subject. In order to compensate

for compression or expansion which might occur during warping of images to match the template, GM maps were "modulated" by multiplying the intensity of each voxel in the final images by the Jacobian determinant of the transformation, corresponding to its relative volume before and after warping [44-45]. GM volumes were computed from these probabilistic images for every subject. Then we calculated the mean of GM volumes (mGMvol) into each group separately, and, finally, for each subject the right and the left hippocampal volumes were normalized separately as follow:

$$\text{Normalized hippocampal volume} = \frac{\text{(raw hippocampal volume x mGMvol)}}{\text{individual GMvol}}$$

The volumes of the perirhinal, entorhinal and parahippocampal cortices depend on the length of the collateral sulcus (COS) [41,43]. Therefore, to keep in account this bias we first calculated the length of each portion of the COS (for the perirhinal cortex = COS_{PERI}; for the entorhinal cortex = COS_{ENT}; for the parahippocampal cortex= COS_{PARA}) by using MANGO. Then, we performed six different linear regressions to regress the length of each portion of the COS from the volumes of the correspondent cortex. Given the high autocorrelation between cortex and correspondent COS, the unstandardized Durbin-Watson residuals were retained for further analyses. Negative residual indicated that the observed cortical volume was smaller than predicted according with the length of the COS. Conversely, positive residual meant that the observed cortical volume was equal or bigger than predicted.

We performed MANOVAs Group (a-MCI *vs*. AD *vs*. HS) by Side (Left *vs*. Right) to assess significant differences in the volumes of the hippocampus and parahippocampal gyrus (perirhinal, entorhinal and parahippocampal cortices, in turn). Moreover, to isolate the effect of the CR level, both for sCR and dCR indexes, we assessed in each group separately a MANOVA CR (High CR *vs*. Low CR) by Side (Left *vs.* Right) to assess significant differences in the volumes of the hippocampus and parahippocampal gyrus (perirhinal, entorhinal and parahippocampal cortices, in turn).

Impact of sCR and dCR indexes, on memory performances and on hippocampal and parahippocampal atrophy

In order to assess the impact of sCR and dCR indexes on the memory deficits together with the atrophy of MTL structures, the mediation effect was estimated by using a series of mediation analyses (based on multiple regression models, performed by using PROCESS a tool of SPSS). The volumes of the hippocampus and of the cortices of the parahippocampal gyrus bilaterally were considered as independent variables, memory performances (15-Word List Immediate and 15-min Delayed recall; Short Story test Immediate and 20-min Delayed recall; Complex Rey's Figure Immediate and 20-min Delayed recall) were considered as dependent variables, and sCR and dCR were considered as mediator or covariate of no interest in turn.

Results

Demographic and clinical characteristics of studied subjects

As reported in Table 1 panel A when considering the whole sample, both groups of patients were significantly older and less educated than healthy subjects ($F_{2,114}$=12.8, p<0.001 and $F_{2,114}$=10.1, p<0.001, respectively). There were also significant differences in the MMSE scores ($F_{2,114}$=74.5, p<0.0001) and in the MTA scale ($F_{2,114}$=54.7, p<0.0001) among all groups. Conversely, there was no statistical differences in gender distribution (AD *vs*.HS: Chi^2=0.63, d.f.=1, p=0.43; AD *vs*. a-MCI: Chi^2=0.01, d.f.=1, p=0.92; a-MCI . HS: Chi^2=0.79, d.f.=1, p=0.37). Moreover, there were significant differences in the CDR ($F_{1,62}$=7.12, p<0.001) and IADL ($F_{1,62}$=21, p<0.001) between AD and a-MCI patients.

Table 1 panel B shows the demographic characteristics of participants divided according to

level of sCR index. Differences between high and low CR were considered within each group. We observed significant differences only in the a-MCI group. In particular, a-MCI patients with low CR were older than those with high CR ($F_{1,38}$= 4.33, p=0.04), there were more females than males (a-MCI: Chi^2=7.52, d.f.=1, p=0.01), and they showed significantly lower MMSE scores ($F_{1,38}$= 6.56, p<0.01). No statistical differences were observed between high *vs.* low CR AD or HS individuals.

Table 1 panel C shows the demographic characteristics of participants divided according to level of dCR index. In the HS group there is a remarkable imbalance between subjects with high (35 subjects) and low (3 subjects) dCR, and as a consequence, we excluded the group from further statistical analyses.

There were no significant differences in the demographic features between patients with different dCR level. In both a-MCI and AD groups the patients with low dCR showed MMSE scores significantly lower than patients with high dCR (a-MCI group: $F_{1,38}$= 7.04, p=0.01; AD group: ($F_{1,37}$= 23.95, p=0.001).

It is remarkable that sCR and dCR indexes differently classified participants as having high or low CR. In particular, in the a-MCI group, sCR index classified 23 patients as having low CR and 17 as having high CR, while the dCR index classified 12 patients as having low CR and 28 as having high CR (Chi-square= 6.15, d.f.=1, p=0.013); in the AD group, sCR index classified 20 patients as having low CR and 19 as having high CR, while the dCR index classified 29 patients as having low CR and 10 as having high CR (Chi-square= 4.45, d.f.=1, p=0.035); Finally in the HS group, sCR index classified 28 subjects as having low CR and 10 as having high CR, while the dCR index classified 3 patients as having low CR and 35 as having high CR (Chi-square= 34.0, d.f.=1, p<0.001).

In addition, when considering the matching between sCR and dCR indexes to classify similarly subjects with certain level of CR we observed that in a-MCI group 5 patients (12.5%) were classified as having low and 9 (22.5%) as having high level in both indexes; in AD group 15 patients (38.5%) were

classified as having low and 5 patients (12.8%) as having high level in both indexes; finally in HS group 2 subjects (5%) were classified as having low and 9 (24.5%) as having high level in both indexes.

We found significant negative correlations between sCR and dCR indexes both in a-MCI and HS groups (a-MCI: r=-0.35, p=0.025; HS: r=-0.63, p<0.001). No significant correlation was found in AD patients (r=-0.15, p=0.36).

Dynamic CR index computation

Linear correlation analyses between MMSE scores and demographic and brain variables

The linear correlation analyses showed significant correlations between the raw MMSE scores and the age (r=-0.25, p=0.008), the years of formal education (r=0.39, p<0.0001), the MTA scores for the left (r=0.39, p<0.0001) and the right hippocampus (r=0.38, p<0.0001), respectively. Conversely, there was no significant correlation between MMSE scores and gender (r=-0.13, p=0.15).

Partial Least Squares and linear regression analyses

Four latent variables were extracted by PLS, as reported in the Figure 2, panel A (see also Table 2, panel A). The first latent variable (LT^{1st}) explained the most of the covariance of X (57.4%) and Y (23.0%), and therefore it was retained for further analyses. The VIP index (Figure 2, panel B and Table 2, panel B) and the loadings revealed that years of formal education as well as left and right MTA scores contributed for the mostly in the composition of LT^{1st} variance. Therefore, years of formal education, left and right MTA scores were regressed again from the LT^{1st}. The regression analysis (Table 2, panel C) revealed that left MTA scores and years of formal education entered in the analysis predicting significantly the variance of the LT^{1st}. According to the Methods, the standardized residual values of the LT^{1st} were considered a proxy of dynamic CR (dCR).

Insert Figure 2 around here

Linear correlation analyses between dCR index and demographical and brain variables

There were no significant correlations between the dCR index and age (r=-0.07, p=0.44), years of formal education (r=-0.01, p=0.97), left and right MTA scores (r=0.001, p=0.95; r=0.03, p=0.75, respectively).

Neuropsychological results

When considering the whole sample, we observed the expected neuropsychological profile with AD patients showing the worst performances in all cognitive domains compared both to a-MCI and HS groups. Patients with a-MCI showed significantly lower scores in memory tests compared to HS (see supplementary Table S1). When considering the groups divided according to their sCR level, there were no significant differences within groups in all neuropsychological tests, with the only exception for the Modified Card Sorting Test. In this test a-MCI patients with low sCR showed significantly worse performance than a-MCI patients with high sCR ($F_{1,38}$=10.5, p=0.003). No further differences were detected (see Table S2). When considering the dCR level no significant differences were detected within groups (see Table S3).

MRI

Hippocampal volumes

As reported in Figure 3 panel A (and in the Supplementary table S4) when considering the whole sample, we observed the typical pattern of distribution of hippocampal volumes among diagnostic groups. Specifically, a two-way ANOVA (Group x Side) revealed a significant effect of Group ($F_{2,114}$=26.2, p=0.001). Post hoc analyses revealed that patients with AD showed significantly smaller

hippocampi than a-MCI patients and HS ($p<0.001$ in both comparisons). Conversely, patients with a-MCI showed no significant difference in the hippocampal volumes in comparison to HS ($p=0.999$). Side effect ($F_{1,114}=1.18$, $p=0.28$) and Interaction ($F_{2,114}=0.68$, $p=0.50$) were not significant. Dividing the groups according to the sCR index (Figure 3, panel B, and Supplementary table S5), a two-way ANOVA (sCR x Side) revealed a significant effect of sCR in the AD patients in the bilateral hippocampus ($F_{1,37}=7.09$, $p=0.011$). Side effect ($F_{1,37}=0.01$, $p=0.902$) and Interaction ($F_{1,37}=0.01$, $p=0.890$) were not significant.

In the a-MCI patients and HS group two separate ANOVAs (sCR x Side) failed to reveal any significant difference. Specifically, in a-MCI patients: sCR effect: $F_{1,38}=0.01$, $p=0.917$; Side effect $F_{1,38}=0.24$, $p=0.620$; Interaction $F_{1,38}=0.50$, $p=0.482$; in HS group: sCR effect: $F_{1,36}=1.93$, $p=0.172$; Side effect $F_{1,36}=1.24$, $p=0.273$; Interaction $F_{1,36}=0.97$, $p=0.329$.

When considering the dCR index (Figure 3, panel C, and Supplementary table S6) in AD patients a two-way ANOVA (dCR x Side) revealed a significant dCR effect in the hippocampal volumes bilaterally ($F_{1,37}=7.09$, $p=0.011$). Side effect ($F_{1,37}=0.06$ $p=0.798$) and Interaction ($F_{1,37}=0.09$, $p=0.756$) were not significant.

In a-MCI patients a two-way ANOVA (dCR x Side) failed to reveal significant effect of dCR ($F_{1,38}=2.69$, $p=0.108$), or Side ($F_{1,38}=1.54$, $p=0.696$), while Interaction almost reached the significance level ($F_{1,38}=3.72$, $p=0.06$) due to a-MCI patients with high dCR level that showed, as revealed by the planned comparisons a significant volume reduction in the left hippocampus ($F_{1,38}=4.28$, $p=0.04$) in comparison to those with low dCR .

Insert Figure 3 around here

Volumes of the perirhinal, entorhinal and parahippocampal cortices

When considering AD, a-MCI and HS groups without differentiating for CR level, in the

parahippocampal gyrus we observed the same pattern found in the hippocampus. In particular, when considering the whole sample there were significant main effects of Group (AD *vs.* a-MCI *vs*. HS) for perirhinal ($F_{2,114}$=10.9, p<0.001), entorhinal ($F_{2,114}$=35.4, p<0.001) and parahippocampal ($F_{2,114}$=10.6, p<0.001) cortices (Figure 4, panel A, and Supplementary table S4). In all cases patients with AD showed reduced volumes compared to HS (p=0.001, in all comparisons), but not compared to a-MCI patients (perirhinal cortex: p=0.405; entorhinal cortex: p=0.321; parahippocampal cortex: p=0.787). Moreover, a-MCI patients showed smaller volumes than HS group in all cortices (perirhinal cortex: p=0.005; entorhinal cortex: p<0.001; parahippocampal cortex: p<0.001). When using the sCR (Figure 4, panel B, and Supplementary table S5) index we did not find significant main effect of sCR (perirhinal cortex : $F_{1,37}$=0.640, p=0.429; entorhinal cortex: $F_{1,37}$=0.758, p=0.390; parahippocampal cortex: $F_{1,37}$=0.582, p=0.451), Side (perirhinal: $F_{1,37}$=0.183, p=0.672; entorhinal: $F_{1,37}$=0.388, p=0.537; parahippocampal cortex: $F_{1,37}$=0.060, p=0.809), or Interaction (perirhinal: $F_{1,37}$=0.469, p=0.498; entorhinal: $F_{1,37}$=0.005, p=0.946; parahippocampal cortex: $F_{1,37}$=0.053, p=0.819), in patients with AD. When considering the a-MCI patients in the perirhinal cortex we observed no significant main effect of sCR ($F_{1,38}$=0.684, p=0.413) or Side ($F_{1,38}$=0.028, p=0.868), but a significant Interaction was detected ($F_{1,38}$=4.527, p=0.05). This Interaction was due to a-MCI patients with high sCR that showed smaller perirhinal volumes in the right hemisphere than to the left ones, conversely no difference was detected in patients with low sCR level. In the entorhinal cortex a-MCI patients did not show a significant main sCR effect ($F_{1,38}$=2.193 p=0.147). Conversely, they showed a significant Side effect ($F_{1,38}$=6.154 p=0.05), due to a smaller volume in the right entorhinal cortex. In addition, we observed also a significant Interaction ($F_{1,38}$=7.130 p=0.05) due to a-MCI patients with high sCR that showed smaller entorhinal volumes in the right hemisphere than to the left ones, conversely no difference was detected in patients with low sCR level. In the parahippocampal cortex there was a significant main Group effect ($F_{1,38}$=10.291 p=0.005) because of patient s with low sCR showed reduced volumes compared to

those with high sCR. There were not significant Side effect or Interaction ($F_{1,38}$=0.247 p=0.622; $F_{1,38}$=1.024 p=0.318, respectively). Nevertheless in HS group we did not find significant main effect of sCR (perirhinal cortex : $F_{1,36}$=0.389, p=0.537; entorhinal cortex: $F_{1,36}$=0.758, p=0.390; parahippocampal cortex: $F_{1,36}$=0.001, p=0.975), Side (perirhinal: $F_{1,36}$=0.247, p=0.623; entorhinal: $F_{1,36}$=0.794, p=0.252; parahippocampal cortex: $F_{1,36}$=0.307, p=0.584), or Interaction (perirhinal: $F_{1,36}$=2.330, p=0.137; entorhinal: $F_{1,36}$=0.252, p=0.619; parahippocampal cortex: $F_{1,36}$=0.055, p=0.816). Figure 4 panel C and Supplementary table S6 illustrated the results according the dCR index. In AD patients we did not find significant dCR effect (perirhinal cortex : $F_{1,37}$=1.039, p=0.315; entorhinal cortex: $F_{1,37}$=0.281, p=0.599; parahippocampal cortex: $F_{1,37}$=2.052, p=0.161), Side (perirhinal: $F_{1,37}$=0.027, p=0.870; entorhinal: $F_{1,37}$=0.323, p=0.573; parahippocampal cortex: $F_{1,37}$=0.126, p=0.725), or Interaction (perirhinal: $F_{1,37}$=1.345, p=0.254; entorhinal: $F_{1,37}$=0.001, p=0.971; parahippocampal cortex: $F_{1,37}$=0.083, p=0.775). In a-MCI patients we did not find significant dCR effect ($F_{1,38}$=1.603, p=0.213), Side ($F_{1,38}$=0.018, p=0.895); or Interaction ($F_{1,38}$=0.714, p=0.404) in the perirhinal cortex; there were no dCR effect ($F_{1,38}$=2.739, p=0.106) or Interaction ($F_{1,38}$=1.204, p=0.280) in the entorhinal cortex, however a significant Side effect was observed ($F_{1,38}$=4.706, p=0.05) due to the fact that the right entorhinal cortex was smaller than the left cortex both in patients with high or low dCR level. Instead, in the parahippocampal cortex a-MCI patients showed a significant dCR effect ($F_{1,38}$=6.978, p=0.05) due to bilateral smaller volumes in patients with high dCR than patients with low dCR level. No Side effect ($F_{1,38}$=0.032, p=0.859) or Interaction ($F_{1,38}$=1.438, p=0.238) were detected.

Insert Figure 4 around here

Impact of sCR and dCR indexes on memory performances and hippocampal and parahippocampal atrophy

In order to reduce the inflation due to the high number of comparisons we limited the mediation

analyses only to the groups showing a significant effect of CR on brain volumes. Specifically, mediation analyses were performed in the AD and a-MCI groups, separately.

In the AD patients, when considering the sCR index as mediator (and the dCR index as covariate of no interest) we found a significant mediation effect of sCR on the right perirhinal cortex in producing the performance in the Short Story test (delayed recall) (Indirect effect: -0.14, Lower Limit CI 95%: -0.39; Upper Limit CI 95%: -0.005). Conversely, when considering the dCR index as mediator (and the sCR index as covariate of no interest) we found a significant mediation effect of the left hippocampus on the performance obtained in 15-Word List (immediate recall) (Indirect effect: -0.18, Lower Limit CI 95%: -0.37; Upper Limit CI 95%: -0.011).

In the a-MCI group we found a significant mediation effect of the sCR index on the right parahippocampal cortex on the performance at 15-Word List (immediate recall) (Indirect effect: -0.15, Lower Limit CI 95%: -0.37; Upper Limit CI 95%: -0.006). Finally, we found a significant mediation effect of dCR index on the bilateral entorhinal cortex on the performance in Short Story test (immediate recall) (for the left entorhinal cortex: Indirect effect: -0.19, Lower Limit CI 95%: -0.43; Upper Limit CI 95%: -0.015; for the right entorhinal cortex: Indirect effect: -0.17, Lower Limit CI 95%: -0.39; Upper Limit CI 95%: -0.027). Notably, in all analyses direct effects were not detected.

Discussion

The present study showed for the first time that the different CR indexes are negatively associated among them. This means that a high sCR value corresponds to a low dCR value, and vice-versa. Moreover, when considering the number of patients classified as having high or low CR we observed significant differences in the categorization in high or low CR level depending on which index has been considered (sCR or dCR index, respectively). In particular, in a-MCI and HS groups the sCR index classified as subjects having a high CR a smaller number of subjects that did dCR index, and in AD

group we observed the reverse pattern.

Therefore, it is reasonable to advance that in the healthy aging and in a-MCI patients the changes in the cognitive efficiency are more able to intercept subjects with higher cognitive resources compared to the educational attainment. On the contrary, in AD patients the static index that reflects more crystallised and more time-independent intellectual functions (e.g. semantic knowledge, proficiency, procedural skills), is more able to classify patients with different cognitive resources. Individuals with high or low static CR index according to their high or low educational level can have high or low general cognitive efficiency. As previously showed [9], a-MCI patients with high educational level and high MMSE score (the measure of general cognitive efficiency) converted to AD significantly later than patients with high education and low MMSE, while in the patients with low education did not exist difference in the conversion time to AD among patients with high or low general cognitive efficiency. Being the dCR index a measure of the changes in the general cognitive efficiency, in the present paper we found an opposite association for sCR and dCR indexes. Interestingly, such an association is very solid in the healthy elderly, less strong but present in the a-MCI patients, and totally absent in the AD patients. It is thus reasonable to hypothesize that in the healthy elderly the high general cognitive efficiency impacts extensively on the brain resilience and compensates better the low educational level, and that this trend is progressively lost from healthy aging to full-blown AD.

However, it is remarkable that in a certain percentage of individuals both indexes are able to classify similarly the subjects. In particular, sCR and dCR indexes were more in accordance for detecting individuals with high CR in the a-MCI and HS group; conversely, they were more in accordance to detect patients with low CR in AD group. We hypothesised that subjects receiving the same level of CR independently from the index considered presented truly that level of reserve. On the contrary subjects changing the level of reserve were in a borderline situation. We retain the more stable subjects a very interesting subgroup and further study focalise on these individuals are needed. Unfortunately, in

the present study this subgroup of subjects presents a very small sample-size, therefore we can not analyse them separately.

The idea that static and dynamic indexes may represent different CR storages is supported also by the observation that the different indexes showed different ability to capture brain volumes changes in the diverse disease stages. In particular, sCR index captured changes in hippocampal volumes between subjects with high and low CR only in the AD patients. In fact, AD patients with low sCR showed smaller volume in the hippocampus bilaterally in comparison to AD patients with high sCR. When considering the effect of sCR on the parahippocampal cortex the same trend was found in the a-MCI patients. However, these findings were not in accordance with the reserve hypothesis. Indeed, the reserve concept assumes that individuals with higher reserve level need to accumulate more neuropathology to express the same clinical symptoms shown by individuals with lower reserve level [1-2]. Thus, we advance that the differences observed in our sample were not only related to a reserve effect but they were likely due to interactions with other factors different from the reserve, such as genetic background or socioeconomic status, or the lifestyle or the work effort that we were unable to assess in our sample. The sCR index probably suffers from the interventions of all these factors unfortunately difficult to disentangle and whose single impact it is hard to ponder.

Conversely, according to the CR hypothesis, the dCR index is able to individuate the different volume changes in hippocampus and parahippocampal cortex in patients with high CR or low CR since a-MCI stage. In fact, a-MCI patients with high dCR showed a higher level of hippocampal and parahippocampal atrophy in comparison to a-MCI patients with low dCR.

It is remarkable that the only dCR index is able to show the volumetric changes in the parahippocampal cortices since a-MCI stage. Notably, these cortices are widely connected with the hippocampus and are involved in the early AD neurodegenerative processes [18]. Studies showed beta-amyloid- [18] and tau-related [46] parahippocampal abnormalities several years before the onset of clinical symptoms

during AD course. Interestingly, the present findings related to dCR index reveal significant differences limited to the parahippocampal cortex, and no significant effect in the perirhinal and entorhinal cortex. A recent study [47] highlighted the different connections and functional role of the cortices of the parahippocampal gyrus. In particular, the perirhinal cortex is part of an anterior temporal network that, through the lateral part of the entorhinal cortex, projects to hippocampus forming the unrefined gist-like representation of objects and non-spatial stimuli [47]. Conversely, the parahippocampal cortex is part of a posterior medial temporal network and it projects by medial part of the entorhinal cortex to hippocampus adding refined details to the cognitive representations [47]. This latter network seems to be particularly vulnerable both to the age-related alterations [47] and to the neurodegeneration [18,46]. On such a basis and considering our present findings, we advance that the changes in the posterior medial temporal network may be precociously detected by using the dCR index.

However, when exploring the relationship among CR indexes, the atrophy of MTL structures and memory performances of patients we found a similar effect in sCR and dCR indexes. Specifically, in the patients with AD sCR index mediated significantly the relationship between the right perirhinal cortex and the performance of immediate recall of the Short Story test while the dCR index mediated significantly the relationship between the left hippocampus and the performance of immediate recall of the 15-Rey's word List test. In the a-MCI patients sCR index mediated significantly the relationship between the right parahippocampal cortex and the performance of immediate recall of the 15-Rey's word List test while the dCR index mediated significantly the relationship between the bilateral entorhinal cortex and the performance of immediate recall of the Short Story test. In all cases the atrophy of MTL structures did not affected the memory performances directly, but through a mediator, sCR or dCR, respectively. Such a mediation implicates that each reduction in the value of the mediator diminishes the memory score for every volumetric change of the MTL structures. It is remarkable that

in patients with AD the static and dynamic CR indexes exerted a mediation effect both in the parahippocampal (namely in the perirhinal cortex) and in the hippocampal structures. Conversely, in a-MCI patients the action of CR indexes on memory functions was shown only in the relationship with the parahippocampal structures. These findings denote that the CR mediates the relationship between brain atrophy and memory performances involving the majority of the MTL structures in the advanced disease stages, while an effect restricted to the parahippocampus was exerted in the early disease stages of the disease, indicating the precocious neurodegenerative process of the posterior medial network. In this context, it should be also recalled that the parahippocampus receives afferent connections from the posterior regions of brain (such as precuneus, posterior cingulate cortex), areas involved in the default mode network (DMN) [48], and, in turn, it projects to the entorhinal cortex and to hippocampus, all these regions playing a key role in the episodic memory system. Even functional MRI studies have indicated the role of the parahippocampus in the modulation of the connectivity into the regions involved in the episodic memory system [49-50]. In addition, parahippocampus connectivity was found to be related to disease progression in AD patients [51].

The present results indicate that static and dynamic CR indexes differently intercept the atrophy of MTL structures, but they similarly modulate the relationship between MTL atrophy and memory performances.

More recently a longitudinal study investigated in a large cohort of individuals followed-up for 20 years the association between cognitive reserve factors and the risk for developing dementia in the presence of brain pathologies [52]. This study highlighted the protective effect exerted by CR revealing that high cognitive reserve was related with a reduction of the risk for developing dementia even in the presence of brain pathology [52].

In conclusion, overall from the literature emerges that the CR hypothesis is currently a hot topic in neuroscience that merits to be extensively investigated. In this viewpoint the present paper contributes

to disentangle some critical aspects highlighting that there is no direct relationship between atrophy of MTL structures and memory dysfunction, as documented by the absence of significant direct effect in the mediation analyses. Conversely, this relationship was significantly mediated by the cognitive reserve. Although these results deserve to be further documented, we here hypothesised that static and dynamic CR indexes are a representation of different warehouses of reserve which do not operate in a parallel but form a more complex system, in which crystallised cognitive abilities and the actual cognitive efficiency interact with brain atrophy impacting on the memory functions.

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Conflict of interests

None of the Authors has any conflict of interest to disclose

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Figure Legends

Figure 1. **Flowchart to compute static and dynamic cognitive reserve indexes in all participants**

Panel A shows the flowchart used to compute sCR in all participants (please see the text for details). Panel B shows the flowchart applied to obtain dCR in the participants. Point B^1 identifies Y (MMSE score) and X (demographic and brain variables); from point B^2 to point B^8 the statistical analyses used to obtain the dCR index are shown (please see the text for details).

Abbreviations: dCR=dynamic Cognitive Reserve index; sCR= static Cognitive Reserve index; MMSE= Mini Mental State Examination; PLS=Partial Least Square analysis; zR= standardised Residuals.

Figure 2. Results of partial least square analyses in all participants

Panel A shows the result of Partial Least Square analysis (). The first latent variable explains most of the covariance of X and Y (57.0% for x and 23.0% for Y). Panel B shows the result of the Variable Importance in the Projection index (VIP index) relatively to the first latent variable. VIP index identifies the education and the left and right MTA scores as variables more contributing to the composition of Mini Mental State Examination score variance into the first latent variable. See text for further details.

Abbreviations: MTA= Medial Temporal Lobe atrophy scale; R=Right; L=Left; VIP= Variable Importance in the Projection index.

Figure 3. Hippocampal volumes

Panel A shows the differences in the volumes of left and right hippocampus in a-MCI, AD and HS groups. Panel B and C show the differences in the volumes of the left and right hippocampus in the

three groups divided according to their sCR (Panel B) and dCR (Panel C). The statistical comparisons between high (in red) vs. low (in blue) static (or dynamic, in turn) Cognitive Reserve level have been performed within each group separately.

Abbreviations: AD= Alzheimer's Disease; a-MCI= amnestic mild cognitive impairment; HS=Healthy Subjects.

See text for further details

Figure 4 Perirhinal, entorhinal and parahippocampal volumes.

Panel A shows the differences in the volumes in the left (in blue) and right (in orange) cortices of the parahippocampal gyrus (encompassing perirhinal, entorhinal and parahippocampal cortex) in a-MCI, AD and HS groups; Panel B and C show the differences in volumes of left (in blue) and right (in orange) cortices of parahippocampal gyrus in the groups divided according to their sCR (panel B) and dCR (panel C). The statistical comparisons high vs. low static (or dynamic, in turn) Cognitive Reserve level have been performed within each group separately.

Abbreviations: AD= Alzheimer's Disease; a-MCI= amnestic mild cognitive impairment; HS=Healthy Subjects; H-CR=High Cognitive Reserve; L-CR=Low Cognitive Reserve.

See text for further details

Table 1. Demographic and clinical characteristics of participants (Mean±SD).

A) Whole sample	a-MCI	AD	HS	p-value <0.05
N	40	39	38	
age [years][a]	69.6±8.3*	71.1±6.7#	62.3±8.4	*a-MCI *vs*. HS #AD *vs*. HS
GENDER (M/F) [b]	16/24	16/23	19/19	
years of formal education[a]	10.0±4.6*	9.3±4.2#	13.2±2.9	*a-MCI *vs*. HS #AD *vs*. HS
MMSE score[a]	27.1±1.9*	20.7±4.5#$	29.3±0.9	*a-MCI *vs*. HS #AD *vs*. HS $AD *vs*. a-MCI
CDR tot	0.6±0.8	1.2±1$	-	$AD *vs*. a-MCI
IADL	7.2±1.2	5.3±1.9$	-	$AD *vs*. a-MCI
MTA	1.9±0.8*	2.6±0.7#$	0.8±0.7	*a-MCI *vs*. HS #AD *vs*. HS $AD *vs*. a-MCI

B) sCR	a-MCI		AD		HS		
	Low	***High***	***Low***	***High***	***Low***	***High***	
N	23	17	20	19	28	10	
age [years][a]	71.8±6.8+	66.5±9.3	72.0±6.6	71.3±7.0	63.0±9.4	60.4±11.7	+ Low *vs*. High
GENDER (M/F)[b]	5/18+	11/6	8/12	8/11	15/13	4/6	+ Low *vs*. High
years of formal education[a]	6.4±1.8+	14.9±2.0	5.7±2.3+	13.1±1.7	11.9±2.7+	16.8±0.6	+ Low *vs*. High
MMSE score[a]	26.5±1.9+	28.0±1.6	19.7 ±3.9	21.7±5.0	29.1±0.9	29.7±0.7	+ Low *vs*. High
CDR tot	0.5±0.0	0.5 ±0.0	1.1±0.8	1.4±1.2	-	-	
IADL	7.4±1.0	6.9±1.5	5.4±2.0	5.3±1.9	-	-	
MTA	1.9±0.8	2.0±0.8	2.5±0.8	2.8±0.6	0.9±0.8	0.8±0.6	

C) dCR	a-MCI		AD		HS		
	Low	***High***	***Low***	***High***	***Low***	***High***	
N	12	28	29	10	3	35	
age [years][a]	67.2±5.7	70.6±9.0	70.6±6.8	74.8±5.7	77.6±2.1	61.0±9.6	
GENDER (M/F) [b]	3/9	13/15	12/17	4/6	3/0	16/19	
years of formal education[a]	11.7±4.7	9.3±4.4	9.3±4.1	9.1±4.9	14.3±2.3	13.1±3.0	
MMSE score[a]	26.0±2.0+	27.6±1.7	19.0±4.0+	25.5±1.4	27.3±1.1	29.4±0.7	+ Low *vs*. High
CDR tot	0.5±0.0	0.5±0.0	1.1±0.7	1.6±1.6	-	-	
IADL	6.9±1.6	7.4±1.0	5.0±1.8+	7.2±1.3	-	-	
MTA	2.1±0.7	1.9±0.9	2.6±0.8	2.7±0.7	1.0±0.0	0.8±0.7	

[a] One-way ANOVA; [b] Chi-square Yates corrected.

Abbreviations: AD= Alzheimer's disease; a-MCI= amnestic Mild Cognitive Impairment; CDR= Clinical Dementia Scale; HS=healthy Subjects; IADL= Instrumental Activity of Daily Living;

MMSE=Mini Mental State Examination; MTA= Medial Temporal lobe Atrophy scale; dCR= dynamic Cognitive Reserve index; sCR= static Cognitive Reserve index.

Table 2. Partial Least Squares analysis in all participants.

Panel A	**Independent variable (X)**		**Dependent variable (Y)**		
Latent factors	**% of Variance**	**% Cumulative**	**% of Variance**	**% Cumulative**	**R^2**
1	**0.57**	**0.57**	**0.23**	**0.23**	**0.22**
2	0.19	0.77	0.01	0.24	0.22
3	0.20	0.97	0.00	0.24	0.22
4	0.02	1.0	0.00	0.24	0.21

Panel B	**VIP index**	**B-matrix**	**Weight**	**Loadings**
Age	0.69	-0.03	-0.34	-0.37
Education	**1.07**	0.29	0.54	**0.44**
L MTA	**1.09**	0.00	0.55	**0.58**
R MTA	**1.08**	0.00	0.54	**0.58**

Panel C	**Unstandardized coefficients**		**Standardized coefficients**			**95% Confidence Interval for B**	
Model	**B**	**Std. Error**	**Beta**	**t**	**p-level**	**Lower Bound**	**Upper Bound**
2 (Constant)	-2.278	0.441		-5.164	0.000	-3.15	-1.40
L MTA	0.001	0.001	0.298	3.433	0.001	0.000	0.001
Education	0.067	0.020	0.290	3.342	0.001	0.027	0.107

Table S1. Performance of a-MCI, AD and HS groups on neuropsychological tests.

Domain	Test	a-MCI	AD	HS
Verbal episodic memory				
	15-Rey's words List:			
	Immediate recall (cut-off ≥ 28.5)	30.1± 4.0*	22.3±8.3#$	45.9±8.5
	Delayed recall (cut-off ≥ 4.6)	4.8 ± 2.2*	2.8±2.6#$	9.7±2.1
	Short Story test:			
	Immediate recall (cut-off ≥ 3.1)	4.4±1.8*	2.2±2.1#$	6.2±1.3
	Delayed recall (cut-off ≥ 2.8)	3.9±2.4*	1.1±2.0#$	6.2±1.3
Visuo-spatial episodic memory				
	Rey's Complex Figure:			
	Immediate recall (cut-off ≥ 6.4)	11.2±7.3	6.7±5.6#	15.7±6.8
	Delayed recall (cut-off ≥ 6.3)	10.8±6.7	5.9±5.2#	14.8±5.8
Verbal short-term memory				
	Digit Span forward (cut-off ≥ 3.7)	5.1±0.9	4.6±1.1#	5.9±1.1
	Digit Span backward	3.6±1.3*	2.7±1.7#	4.6±0.9
Visuo-spatial short-term memory				
	Corsi Span forward (cut-off ≥ 3.5)	4.4±0.5	3.2±1.5#$	5.0±0.9
	Corsi Span backward	3.8±1.2	2.5± 2.0	4.2±0.8
Executive functions				
	Phonological Word Fluency (cut-off ≥ 17.3)	31.3±7.6	20.3±10.4#$	36.8±9.1
	Modified Card Sorting Test			

	Criteria achieved (cut-off ≥ 4.2)	4.5±1.7	1.8±1.3[#$]	5.8±0.7
Language				
	Naming of objects (cut-off ≥ 22)	28.1±2.0	22.9±9.0[#$]	29.2±1.0
Reasoning				
	Raven's Coloured Progressive Matrices (cut-off ≥ 18.9)	27.8±3.9	19.0±9.0	31.5±4.5
Constructional praxis				
	Copy of drawings (cut-off ≥ 7.1)	9.4±1.5	5.8±4.0[#]	11±1.0
	Copy of drawings with landmarks (cut-off ≥ 61.8)	65.0±11.0	47.8±22.9[#$]	66.4±2.8
	Rey's Complex Figure-Copy (cut-off ≥23.7**)**	30.4±5.9	16.5±16.3	32.2±4.0

* a-MCI*vs*. HS; #AD *vs*.HS; $ AD *vs*. a-MCI; all p≤0.003 after Bonferroni's correction

Abbreviations: AD = Alzheimer's disease; a-MCI= amnestic Mild Cognitive Impairment; HS = Healthy Subjects.

Table S2. Performance obtained by participants divided according the sCR level on neuropsychological testing.

sCR	Participants					
	a-MCI		AD		HS	
	Low	*High*	*Low*	*High*	*Low*	*High*
Neuropsychological test						
Verbal episodic memory						
15-Word List:						
Immediate recall (cut-off ≥ 28.5)	31.5±6.4	28.2±4.9	23.1±6.4	21.4±7.1	46.9±8.6	43.3±8.1
Delayed recall (cut-off ≥ 4.6)	5.6±2.5	3.7±1.2	2.8±2.8	2.8±2.5	9.7±2.1	9.9±2.7
Short Story:						
Immediate recall (cut-off ≥ 3.1)	4.2±1.9	4.8±1.7	2.0±2.1	2.4±2.1	5.9±1.3	7.0±0.6
Immediate recall (cut-off ≥ 2.8)	3.8±2.7	3.9±2.0	0.8±1.8	1.4±2.2	5.9±1.2	6.7±0.7
Visuo-spatial episodic memory						

Rey's Complex Figure						
Immediate recall (cut-off ≥ 6.4)	11.5±5.2	10.9±9.5	5.9±5.9	7.4±5.4	15.3± 7.3	16.7±5.5
Delayed recall (cut-off ≥ 6.3)	11.3±6.5	10.3±7.0	6.1±5.8	5.6± 4.7	14.0±6.1	16.9±4.4
Verbal short-term memory						
Digit Span forward (cut-off ≥ 3.7)	4.8±0.8	5.5±0.9	4.8±1.1	4.4±1.2	6.1±1.1	5.4±1.0
Digit Span backward	3.2±1.4	4.0±0.8	2.5±1.7	2.8±1.5	4.5±0.8	5.0±1.0
Visuo-spatial short-term memory						
Corsi Span forward (cut-off ≥ 3.5)	4.5±0.5	4.4±0.6	3.2±1.6	3.2±1.5	5.1±0.8	4.7±1.0
Corsi Span backward	3.5±1.4	4.0±0.9	2.1±1.8	2.7±2.2	7.8±9.3	8.8± 0.8
Executive functions						
Phonological verbal fluency (cut-off ≥ 17.3)	30.1±8.4	31.8± 6.6	22.3± 11.0	18.3±10.0	37.0±7.7	35.3± 13
Modified Card Sorting Test						
Criteria achieved (cut-off ≥ 17.3)	3.7± 1.7*	5.5±1.2	1.7±1.0	2.0±1.6	5.8±0.7	5.8±0.4

Reasoning						
Raven's Progressive Matrices (cut-off ≥ 18.9)	27.0±4.1	28.8±6.4	19.6±9.2	18.3±9.1	31.4±6.2	31.9±3.1
Language						
Naming of objects (cut-off ≥ 22)	27.4± 2.3	29.1±1.1	21.3±9.5	24.7±8.2	29.0±1.1	29.6±0.8
Constructional praxis						
Copy of drawings (cut-off ≥ 7.1)	9.1±1.5	9.8±1.4	5.2±3.8	6.4±4.3	10.1± 1.3	10.7± 1.3
Copy of drawings with landmarks (cut-off ≥ 61.8)	62.9±4.3	67.7±1.3	40.4±4.0	55.6±9.1	65.6±1.0	68.7±1.3
Rey's Complex Figure-Copy (cut-off ≥23.7**)**	29.1±6.9	32.1±3.9	15.7±14.7	17.2±12.4	31.5±4.6	33.9±1.4

Post-hoc comparisons: * Low *vs.* High sCR. All p≤0.003 after Bonferroni's correction

Abbreviations: AD = Alzheimer's disease; a-MCI= amnestic Mild Cognitive Impairment; HS = Healthy Subjects.

Table S3. Performance obtained by participants divided according the dCR level on neuropsychological testing.

dCR	Participants			
	a-MCI		AD	
	Low	***High***	***Low***	***High***
Neuropsychological test				
Verbal episodic memory				
15-Word List:				
Immediate recall (cut-off ≥ 28.5)	29.0±4.8	30.6±6.5	20.3±8.1	28.8±5.2
Delayed recall (cut-off ≥ 4.6)	4.0±2.0	2.2±2.2	2.4±2.5	4.2±2.8
Short Story:				
Immediate recall (cut-off ≥ 3.1)	3.5±2.1	4.8±1.6	2.4±2.2	1.6±2.0
Immediate recall (cut-off ≥ 2.8)	3.2±2.2	4.1±2.4	1.1±2.1	1.1±1.9

Visuo-spatial episodic memory				
Rey's Complex Figure				
Immediate recall (cut-off ≥ 6.4)	9.6±9.8	11.9±6.1	7.1±5.7	5.9±5.7
Delayed recall (cut-off ≥ 6.3)	8.8±6.2	11.6±6.8	5.4±5.2	6.9± 5.2
Verbal short-term memory				
Digit Span forward (cut-off ≥ 3.7)	5.4±1.0	5.0±0.9	4.9±1.0	4.9±1.3
Digit Span backward	3.7±0.7	3.5±1.4	2.4±1.6	3.3±1.6
Visuo-spatial short-term memory				
Corsi Span forward (cut-off ≥ 3.5)	4.1±0.2	4.5±0.6	3.0±1.5	3.9±0.9
Corsi Span backward	3.7±0.6	3.8±1.4	2.0±1.9	4.0±2.4
Executive functions				
Phonological verbal fluency (cut-off ≥ 17.3)	33.0±7.8	30.6± 7.6	18.7± 10.2	25.6±9.8
Modified Card Sorting Test				

Criteria achieved (cut-off ≥ 17.3)	4.8± 1.6	4.4±1.8	1.9±1.4	1.6±1.3
Reasoning				
Raven's Progressive Matrices (cut-off ≥ 18.9)	28.5±4.5	27.5±3.6	18.1±8.5	21.6±10.7
Language				
Naming of objects (cut-off ≥ 22)	29.2± 0.1	27.7±2.2	21.4±9.8	27.2±2.3
Constructional praxis				
Copy of drawings (cut-off ≥ 7.1)	9.5±1.7	9.3±1.4	5.3±3.9	7.4±4.4
Copy of drawings with landmarks (cut-off ≥ 61.8)	67.7±1.8	63.8±1.3	44.3±24.0	59.9±13.7
Rey's Complex Figure-Copy (cut-off ≥23.7**)**	28.8±5.4	31.1±6.0	15.4±12.5	19.4±15.9

Post-hoc comparisons: * Low *vs.* High dCR. All p≤0.003 after Bonferroni's correction

Abbreviations: AD = Alzheimer's disease; a-MCI= amnestic Mild Cognitive Impairment; HS = Healthy Subjects.

Table S4. Mean and standard deviation of hippocampus and of the cortices of the parahippocampal gyrus in the entire sample

Whole sample	a-MCI	AD	HS
L Hippocampus[a]	2863.5±541.8	2307.4±386.3	2851.8±334.3
R Hippocampus[a]	2869.1±509.6	2316.0±451.5	2907.8±385.4
L Perirhinal cortex[b]	-46.8±314.6	-126.1±300.7	194.7±485.9
R Perirhinal cortex[b]	-37.9±301.6	-141.4±214.1	201.6±381.6
L Entorhinal cortex[b]	-22.5±121.7	-62.9±100.3	96.2±144.5
R Entorhinal cortex[b]	-46.7±98.2	-70.2±89.6	132.1±120.8
L Parahippocampal cortex[b]	-73.7±222.4	-41.6±211.8	131.1±253.2
R Parahippocampal cortex[b]	-56.9±181.9	-34.3±160.6	103.6±181.4

[a]Volumes expressed in mm^3; [b]Volumes expressed as residuals

Abbreviations: AD = Alzheimer's disease; a-MCI= amnestic Mild Cognitive Impairment; HS = Healthy Subjects; L=Left; R=Right.

Table S5. Mean and standard deviation of hippocampus and of the cortices of the parahippocampal gyrus in the sCR sample

sCR	Participants					
	a-MCI		AD		HS	
	Low	*High*	*Low*	*High*	*Low*	*High*
L Hippocampus[a]	2791.3±477.3	2961.2±620.1	2211.3±6.4419.9	2382.±415.7	2850.7±295.2	2855.4±456.8
R Hippocampus[a]	2827.2±466.2	2925.7±573.0	2247.9±480.9	2357.9±415.7	2918.3±362.5	2875.2±472.6
L Perirhinal cortex[b]	-104.1±277.7	35.5±354.2	-109.6±306.6	-135.3±309.2	176.4±539.2	238.5±346.3
R Perirhinal cortex[b]	-22.9±340.2	-59.6±244.7	-97.3±183.5	-188.5±243.9	230.5±416.1	132.2±298.5
L Entorhinal cortex[b]	-55.8±91.0	25.4±145.7	-49.5±91.2	-72.7±111.4	98.5±139.7	90.7±163.0
R Entorhinal cortex[b]	-53.5±78.3	-36.9±123.6	-57.9±87.2	-83.3±95.2	143.9±134.4	103.4±77.9
L Parahippocampal cortex[b]	-156.4±200.9	45.0±201.4	-65.2±234.1	-16.3±195.8	134.7±254.5	122.5±263.7
R Parahippocampal cortex[b]	-118.9±184.8	32.2±139.2	-50.1±141.8	15.8±184	101.4±188.	108.9±171.4

[a]Volumes expressed in mm^3; [b]Volumes expressed as residuals

Abbreviations: AD = Alzheimer's disease; a-MCI= amnestic Mild Cognitive Impairment; HS = Healthy Subjects; L=Left; R=Right.

Table S6. Mean and standard deviation of hippocampus and of the cortices of the parahippocampal gyrus in the dCR sample

dCR	Participants			
	a-MCI		AD	
	Low	***High***	***Low***	***High***
L Hippocampus[a]	2898.7±519.1	2848.4±559.8	2360.6±387.0	2104.0±313.6
R Hippocampus[a]	2785±543.5	2904.8±500.4	2351.1±477.1	2157.5±328.1
L Perirhinal cortex[b]	64.2±335.3	-96.1±298.2	-127.9±307.4	-106.3±309.8
R Perirhinal cortex[b]	18.8±394.8	-63.2±254.7	-180.9±168.9	-35.8±298.1
L Entorhinal cortex[b]	28.1±113.3	-44.9±120.5	-65.5±96.4	-48.2±116.4
R Entorhinal cortex[b]	-17.2±90.5	-59.8±100.2	-74.6.1±95.1	58.5±81.5
L Parahippocampal cortex[b]	59.1±258.1	-132.8.1±179.8	-15.3±230.8	-111.8± 151.6
R Parahippocampal cortex[b]	32.3±190.6	-96.63±166.4	-12.9±179.2	-88.8±91.7

[a]Volumes expressed in mm^3; [b]Volumes expressed as residuals

Abbreviations: AD = Alzheimer's disease; a-MCI= amnestic Mild Cognitive Impairment; HS = Healthy Subjects; L=Left; R=Right.

Figure 1. Flowchart for the computation of the static and dynamic cognitive reserve indexes

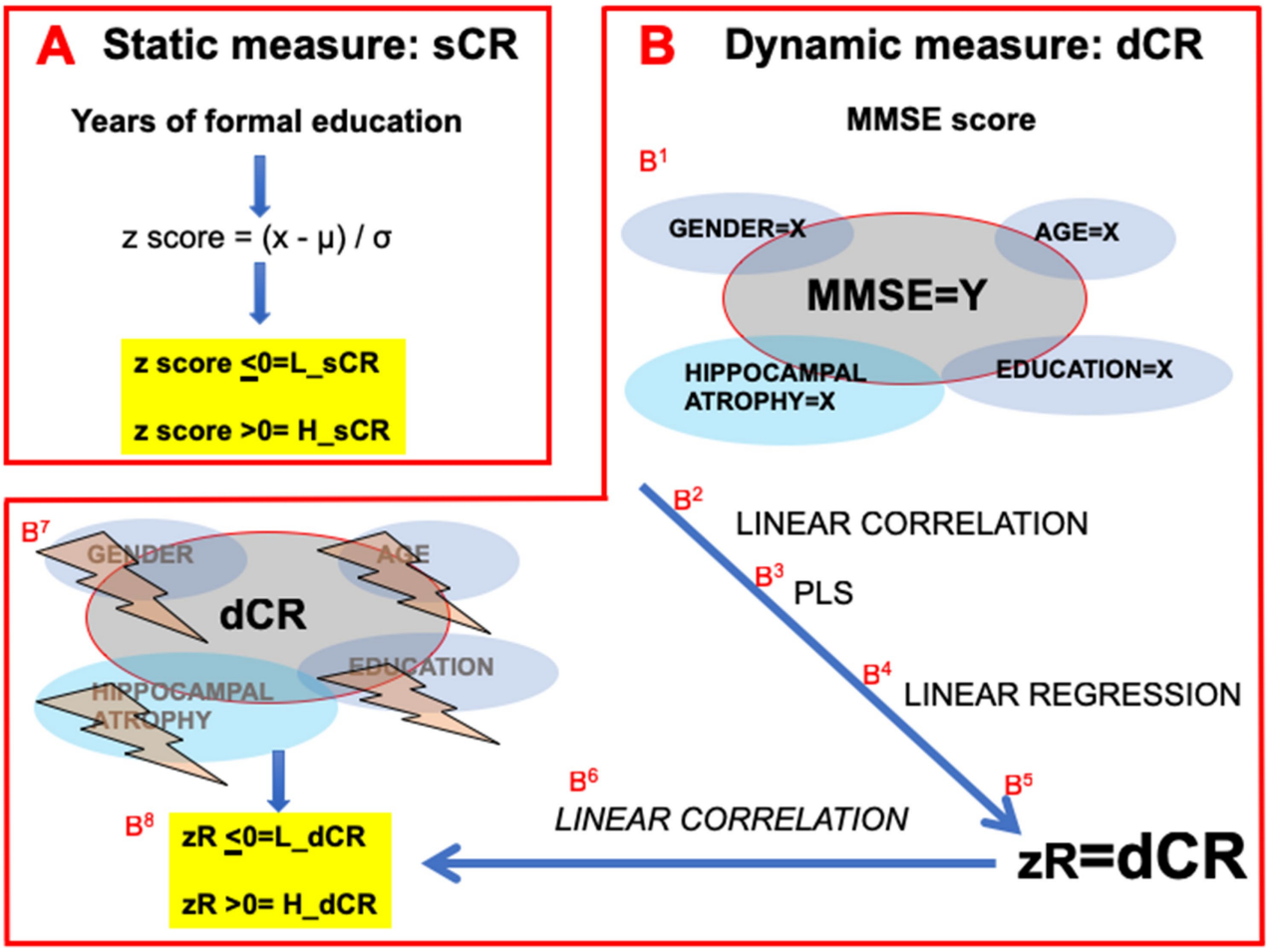

Figure 2. Partial Least Square analysis

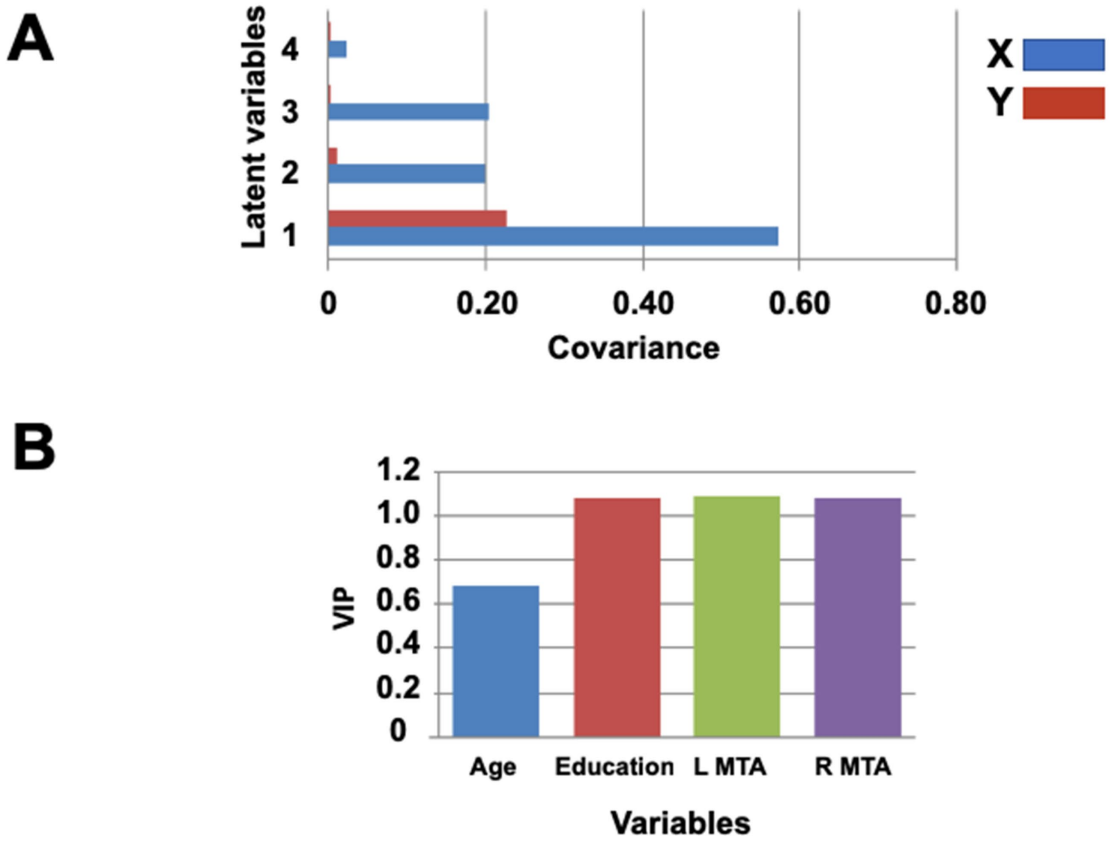

Figure 3. Hippocampal volumes

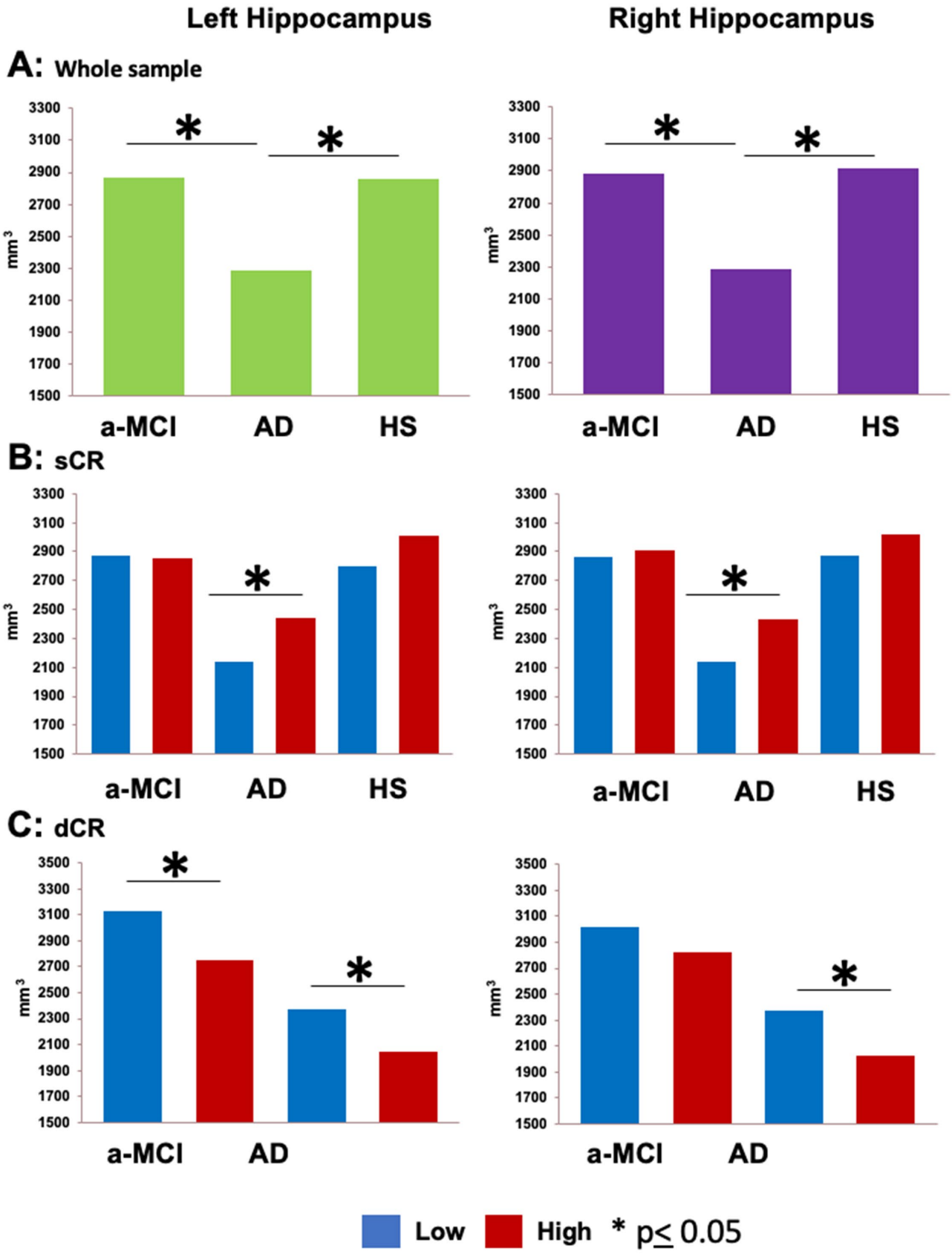

Figure 4. Volumes of the cortices in the parahippocampal gyrus